

Profiling Nutritionally Important Carotenoids from Genetically-Diverse Tomatoes by IR Spectroscopy



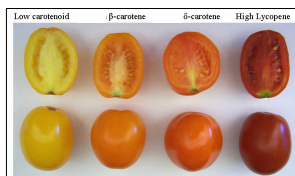
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Introduction & Objectives

- Epidemiological studies have indicated a direct relationship between a high dietary intake of carotenoids and a decreased risk of certain types of cancer, cardiovascular diseases, and age-related macular degeneration (Bhosale et al. 2004).
- Tomato is an excellent candidate for the development of functional foods due to its role as the major source of dietary carotenoids in North America.
- Carotenoid analysis currently relies heavily on HPLC (Barba et al. 2006). Although reliable and accurate, this method is time-consuming, requires extensive sample preparation, and uses and disposes of hazardous organic solvents.
- Rapid profiling of carotenoids in tomato can facilitate the selection of new plant breeding lines and the processing of carotenoid-rich foods.
- **THE OBJECTIVE** of this research was to develop a rapid and efficient protocol for profiling tomato carotenoids using infrared (IR) spectroscopy.

Materials & Methods

Tomato breeding lines (24) were grouped in 8 classes* based on their different carotenoid metabolism.

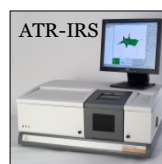


Hexane was used to extract the lipid fraction from samples (Nguyen and Schwartz, 1998).

Lipid fraction

*High *trans*-lycopene, β -carotene, δ -carotene, *tangerine*, *tangerine virescent*, *albacaca-tangerine*, β -carotene-tangerine, and low-carotenoid.

Lipid fractions were directly applied onto an ATR (attenuated total reflectance) crystal plate for spectra acquisition and injected in a HPLC system for carotenoid separation.



Multivariate analysis (SIMCA) was used to classify lipid fractions based on infrared spectral signatures. Results were compared with data from HPLC.

Results & Discussion

- The strong contribution of carotenoids to the overall IR spectra of tomato lipid fractions permitted the identification of characteristic infrared bands for each of the 8 genotype groups. The second derivative of the IR spectra (Fig 1) enabled SIMCA to generate models that differentiated each genotype group based on their carotenoid profile. Class projections allowed tridimensional visualization of clustering patterns. Discriminatory bands at 957cm^{-1} and 964cm^{-1} (*trans*-C-H out-of-plane vibrations) had a predominant role in distinguishing the chemical structure of the 8 groups (Fig 2).

Fig 1: Second Derivative of IR spectra

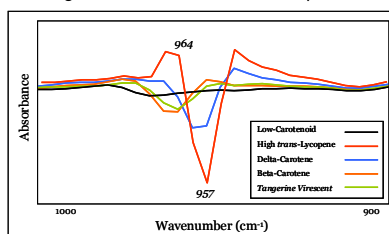


Fig 2: Discriminating power graph

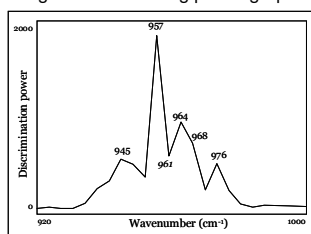
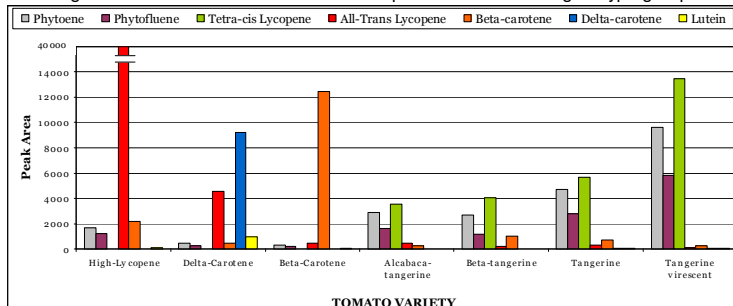


Table 1: SIMCA Interclass distances** among 8 variety groups.

	Tangerine Virescent	Beta-Carotene	Beta-tangerine	Delta-carotene	Tangerine	Low-carotenoid	High trans-lycopene
Beta-Carotene	10.7						
Beta-tangerine	9.3	4.8					
Delta-carotene	10.4	12.9	11.9				
Tangerine	7.7	5.6	3.1	11.3			
Low-carotenoid	15.6	9.4	8.7	13.8	8.1		
Trans-lycopene	21.8	22.0	19.4	12.2	22.0	21.0	
Albacaca-tang.	14.4	7.3	4.2	14.4	4.2	8.0	29.4

Fig 3: Relative carotenoid content in the lipid fractions of the 8 genotype groups



- Interclass (IC) distances produced by the SIMCA model (Table 1) correlated well with the HPLC carotenoid profiles (Fig 3). Smaller IC distances were associated to classes with similar carotenoid composition (*tangerine*, β -*tangerine* and *albacaca-tangerine*) while large IC distances were related to groups with more particular carotenoid profiles (high *trans*-lycopene, *tangerine virescent*, β -carotene, δ -carotene). Class distances greater than 3.0 are regarded as significant to identify 2 groups of samples as different.

Conclusions

- SIMCA was able to classify each tomato variety by the type and quantity of carotenoids using chemical information collected by ATR-IR spectroscopy. Models exhibited well-separated clusters (IC >3.0) that correlated well with the information obtained by HPLC.
- The functional groups found to be responsible for most of the differentiation of clusters in the SIMCA model, *trans*-C-H out-of-plane bend and its *cis* and *trans* conjugation, indicated that discrimination was primarily based on degree and type of unsaturation in carotenoids.
- In summary, ATR-IR coupled with multivariate analysis has shown to be a robust and fast technique (3 minutes versus 60 minutes in HPLC), providing a valuable profiling tool for carotenoids in tomatoes.

REFERENCES

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